University of Alberta Library
0 1620 3416653 6

For Reference

NOT TO BE TAKEN FROM THIS ROOM

Ex libris
universitatis
albertaensis

Thesis
1948
#21

TABLE OF CONTENTS

	Page
Acknowledgements -	1
Introduction -	2
Materials and Preparation -	4
The Cloacal Protruberance -	7
Structure in Vesper Sparrow -	9
Contents of the Glomeral Duct -	14
Structure in Horned Lark -	15
Discussion -	16
Sperm Smears -	19
Spermatozoa -	23
Vesper Sparrow -	23
Horned Lark -	24
Discussion -	25
Testis -	27
Testis Size of Horned Lark -	27
An Example of Physiological Separation of Subspecies -	29
Sertoli Cells -	32
Summary -	33
Bibliography -	34
Illustrations -	
Fig. 1, Graph	37
Figs. 2 - 16, Drawings	38
Figs. 17 - 25, Photomicrographs	44
Table I -	5
Table II -	21
Table III -	26
Table IV -	30

Digitized by the Internet Archive
in 2018 with funding from
University of Alberta Libraries

https://archive.org/details/certainaspectsof00salt

ACKNOWLEDGEMENTS

* * *

I am deeply indebted to Dr. Wm. Rowan, Head of the Department of Zoology, for assistance and advice during the course of this study; for introduction to the writings or the persons of many ornithologists of note; for willingly undertaking many small, and therefore onerous, commissions during my absence from the University; and for encouragement at all times.

I wish to thank Dr. R. Miller and Dr. W. Hughes of the Department of Zoology, whose advice or critical comments, freely given upon request, served to keep me on the narrow path of truth.

My thanks are also extended to Dr. Emil Witschi of the State University of Iowa for helpful information and encouragement given to one who is not his pupil.

Finally I owe a great debt of gratitude to Mr. Robert Lister, Technician of the Department of Zoology, not only for assistance in the preparation of materials, but for providing an unfailing source of interest and enthusiasm upon which I very often leaned heavily.

CERTAIN ASPECTS
OF THE MALE REPRODUCTIVE SYSTEM
OF SOME PASSERINE BIRDS

* * *

INTRODUCTION

In recent years a great deal of research has been carried out on the reproductive systems of birds, by some workers for its intrinsic value, by others because the sex hormones seem to control much of the birds' behavior including the fascinating and still little-understood act of migration. The fact that birds show a cyclical growth and decline of the gonads which is seasonal, at least in birds of the temperate regions, is now well known. A corresponding fluctuation in some of the accessory organs has more recently been discovered. Both are believed to be hormone-controlled.

Much of this research has been of an experimental nature carried out on birds kept in the laboratory. Passerine birds have been found particularly suitable as experimentals, especially the Fringillids which are small and easily kept in captivity.

Work with living birds in a natural state has been small despite the fact that excellent methods of securing wild birds unharmed have been developed by the bird banders. The reason for this is the necessity of sacrificing specimens in order to determine their stage of sexual development. This is also the most serious obstacle to successful research with laboratory experimentals. A method of determining the condition of the gonads without mutilation or death of the subject would do much toward advancing a study of sex among birds in a natural state.

These facts were well known to the writer long before he started this thesis in the spring of 1946 as an orthodox investigation into the testicular structure and activity of some local species of passerine birds. The work soon became boring as nothing new was uncovered. Consequently it was decided to change the subject of investigation. A search of the literature had shown that a protruberance of the anal region, commonly used by the writer to sex passerine birds, was practically unknown. It also showed that very little was known about the spermatozoa of many species of birds and that most descriptions were taken from specimens found in the testes. The idea of taking sperm samples, although familiar to the poultryman, seemed to be practically unknown to ornithologists. These two related subjects were therefore chosen for investigation. Their study forms the major part of this thesis but a short section on the testis is included to record part of the work done upon that subject.

In this study the cloacal protruberance, an external feature related to the reproductive system, is found to be present on the males of some passerine species. It is believed that it could be used as an indicator of testicular development. A method of taking anal smears from males of some passerine species is also described. It is suggested that the nature of the semen obtained on these smears might be used as an indication of sexual maturity.

It is not the purpose of this thesis to follow the cyclical changes in either the cloacal protruberance or the nature of the semen. This is merely an introductory study, mainly anatomical and histological, which naturally should be followed by accurate quantitative work. Any quantitative facts obtained incidentally to the main problem have, however, been included.

MATERIALS

Three species of passerine birds were selected during 1946 as the major species for study: the horned lark, Otocoris alpestris; the vesper sparrow, Pooecetes gramineus; and the chestnut-collared longspur, Calcarius ornatus. Ordinarily, all are commonly found and easily obtained in the Calgary district. During 1947 the chestnut-collared longspur was locally scarce, and since the specimens collected during the two years did not form a representative series, this species was placed with several others to form a group of minor importance which was used mainly for comparative purposes.

This study, therefore, deals chiefly with the two species, the horned lark and the vesper sparrow. Both are members of the order Passeres but the hornd lark belongs to the family Alaudidae, and the vesper sparrow to the family Fringillidae. Their habitats are similar; both are found typically on the grassy prairie-lands of southern Alberta. They are partially insectivorous and partially granivorous. Both nest on the ground. But while the vesper sparrow arrives in southern Alberta about the end of April and nests only once during the summer, the horned lark arrives about the end of February and produces two broods by the middle of July.

In all, one hundred and forty-six specimens comprising thirty-one species of birds, were examined. Of these, one hundred and thirteen specimens of eleven species, were passerine birds. A list of the species examined, with the number of specimens of each, is given in Table I.

Most of the specimens were shot with light loads of dust-shot to prevent excessive damage. The majority were taken in the immediate vicinity of Calgary, but a few winter horned larks were secured at Rosebud, about fifty miles east and fifteen miles north of Calgary, where horned larks of one subspecies or another may be

TABLE I

SPECIMENS EXAMINED

* * *

Species	No. Females	No. Males	Total
Order: Passeres			
Otocoris alpestris	4	34	38
Pooecetes gramineus	3	23	26
Calcarius ornatus	1	20	21
Passerculus sandwichensis	1	3	4
Molothrus ater		3	3
Euphagus cyanocephalus	1	9	10
Sturnella neglecta		4	4
Agelaius phoeniceus		1	1
Passer domesticus		4	4
Tyrannus tyrannus		1	1
Empidonax trailli	1		1
Order: Anseres			
Anas platyrhynchos		6	6
Dafila acuta		1	1
Order: Ralli			
Fulica americana	2		2
Order: Laro-limicolae			
Pisobia melanotus		4	4
Pisobia bairdi	1	2	3
Pisobia minutilla		1	1
Recurvirostra americana		1 (juv)	1
Catoptrophorus semipalmatus		1	1
Limosa fedoa		1	1
Larus delawarensis		1	1
Larus pipixcan		1	1
Order: Galli			
Bonasa umbellus	1		1
Pedioecetes phasianellus		1	1
Phasianus colchicus		3	3
'Golden pheasant', domesticated		1	1
Order: Accipitres			
Astur atricapillus		1	1
Falco rusticolus	1		1
Falco columbarius	1		1
Order: Striges			
Nyctea nyctea		1	1
Surnia ulula caparoch	1		1
	18	128	146

found during every month of the year. Specimens were taken mainly during the months of April, May, and June. There is a scarcity of material for the months of July and August when my attendance at the University was required. This prevented a study of some of the phases of regression in the reproductive system and the work of others has been drawn upon to some extent to bridge this gap. Since this study is not intended to follow annual cyclical changes, but merely to examine certain parts of the reproductive system during the breeding season, this deficiency is not considered important.

PREPARATION OF MATERIALS

Most of the specimens were examined in the lab within one, or occasionally two, hours of collecting. Here they were dissected, measurements taken, and records and drawings made. Parts required for further examination were fixed in Bouin's solution for a minimum of twenty-four hours and stored in seventy percent alcohol.

Materials for histological study were imbedded in wax following the orthodox procedure. They were then sectioned at a thickness of 10μ and stained with Delafield's haematoxylin and eosin. Serial sections were cut at a thickness of 20μ.

The method of taking sperm smears will be described under that heading. A great variety of methods of preparing smears was used. The most successful proved to be immediate drying with no fixative or stain. Unfortunately this method fails to bring out the nucleus. To show the nucleus Delafield's haematoxylin was used with eosin as a counterstain. It is believed that the use of Bouin's fixative changes the shape of the nucleus but positive proof of this was not obtained.

THE CLOACAL PROTRUBERANCE

When collecting passerine birds the writer has long been accustomed to sex them in the field by an external examination of the cloacal region. In males this region swells considerably prior to the breeding season to form a definite protruberance which undoubtedly assists in coition. The organ persists throughout the breeding season after which it regresses and disappears. Its use as a diagnostic characteristic for sex is therefore confined to the spring and summer months, but at that time it is particularly useful in sexing the sparrows, many of which are alike in breeding plumage. The fact that ornithologists of my acquaintance were not familiar with this organ, and the lack of reference to it in texts and in papers dealing with the avian reproductive system, stimulated this investigation.

The protruberance was observed in breeding males of the following species of passerine birds examined during the course of this study:

Horned lark, Otocoris alpestris

Vesper sparrow, Pooecetes gramineus

Savannah sparrow, Passerculus sandwichensis

Chestnut-collared longspur, Calcarius ornatus

Cowbird, Molothrus ater

Meadowlark, Sturnella neglecta

Brewer's blackbird, Euphagus cyanocephalus

Redwing blackbird, Agelaius phoeniceus

Stresemann (1934) notes it in the American robin, Turdus migratorius, and in the Towhee, Pipilo erythrophthalmus.

Rowan failed to notice the protruberance on the Slate-colored junco, Junco hyemalis, which he used in his experiments on the manipulation of the reproductive cycle in birds by means of light.

Riley has worked extensively on the English sparrow, Passer domesticus, but does not mention the presence of such a protruberance. It is not mentioned by Witschi or Keck who have also studied the reproductive system of this sparrow. Witschi, (personal communication) however, says that it has been observed. Both primary and secondary sex organs of the Starling, Sturnus vulgaris, have been the subject of investigations by Bullough, Bissonnette, and Witschi. Bullough's work in particular treats in great detail with all parts of the reproductive systems of both male and female but, in common with the other writers, he says nothing of a protruberance of the anal region. The writer has observed the cloacal protruberance on the junco and the English sparrow. Unfortunately he has been unable to secure for examination a specimen of the starling which has only recently extended its range into Alberta.

No evidence of a cloacal protruberance was found during this study on the following non-passerine birds used as comparative material:

Laro-limicolae:	Baird's sandpiper, Pisobia bairdi
	Pectoral sandpiper, Pisobia melanotus
	Least sandpiper, Pisobia minutilla
	Marbled godwit, Limosa fedoa
	Franklin gull, Larus pipixcan
Anseres:	Mallard, Anas platyrhynchos
Galli:	Sharp-tailed grouse, Pedioecetes phasianellus.

The godwit, gull, mallard, and grouse were examined while in breeding condition. The sandpipers were migrating birds taken only as late as May 18. At that time, although there was no evidence of a cloacal protruberance (Fig. 4), the testes of these birds were well developed and contained completed sperms in the tubules.

Rowan (1929) records and pictures completed sperms in the testes of a closely related species, the semipalmated sandpiper, Ereunetes pusillus, taken during migration at Beaverhills Lake, Alberta. He has also described (1926) the mating display of this species performed during the month of May. If a cloacal protruberance is produced at all, it must be by very rapid growth during the short period of time required by these sandpipers to reach their breeding grounds. Observations on passerine birds lead to the belief that, as in the testes, growth of the cloacal protruberance is slow at the start and then precipitate. However, there is always some early evidence of it. Since this evidence is lacking in the sandpipers it is assumed, though conclusive evidence is lacking, that a cloacal protruberance is not present on breeding sandpipers of the three species examined.

Structure in Vesper Sparrow

The cloacal protruberance of the vesper sparrow is not fully developed when the bird first appears in the Calgary district about the beginning of May. It is then a cylindrical body about 7-8 mm. in length projecting from the abdomen under the uropygium. (Fig.2). After the middle of May the protruberance becomes bulbous, swelling mainly on its dorsal side. (Fig. 3).

When fully developed the cloacal protruberance of the vesper sparrow is a roughly-spherical body nearly 12 mm. in greatest diameter, protruding backward from the abdomen under the uropygium. Its ventral surface is somewhat flattened. On its distal surface is the anus surrounded by rugose lips devoid of feathers. The long stiff feather group known as the anal tuft surrounds these lips and the remainder of the protruberance bears small contour feathers, down, and filoplumes. From the anus during the breeding season, there often protrudes a small papilla overlapped by the

lips in much the same way as the eyelids fit over the eye. On this papilla is a small opening. The papilla is the everted proctodaeum and the opening the outlet of the urodaeum (Figs. 3,9 & 11).

A dissection of the cloacal protruberance (Fig. 9) shows the rectum entering it near the dorsal surface and opening into the cloaca which extends back medially to the anus. Copradaeum, urodaeum, and proctodaeum are not distinguishable in gross dissection. On each side of the rectum, and running very close to it, are the very fine ureters which enter the sides of the cloaca. The vasa deferentia follow the route of the ureters but change their character before reaching the protruberance. Shortly before entering it each starts to coil and fold upon itself to form a large pear-shape mass, the seminal glomus, whose narrower end points anteriorly. The glomera, separated by the rectum and cloaca, spread over these dorsally and ventrally usually meeting ventrally in the middle line. They remain distinct, however, as two bodies separated by their connective tissue sheaths. A continuation of the tube of which each is composed reappears on the median surface of the glomus toward its posterior end but it is now a thick tube, the ejaculatory duct. The two ejaculatory ducts rum close to the ureters near their ends and enter the urodaeum behind and dorsal to the ureter openings. Their openings are situated fairly close together on the dorsal surface of the cloaca. The identity of these various structures may be obscured by considerable fatty tissue surrounding and separating them. Around the whole of the above group of organs, and thus forming the wall of the cloacal protruberance, is a thin layer of voluntary muscle, the anal sphincter. The walls of the proctodaeum are composed mainly of voluntary muscle, the cloacal sphincter. The proctodaeum may be found in either the inverted or everted position (Figs. 10 & 11).

During May and June the seminal glomera are the largest and most obvious organs in the cloacal protruberance of the vesper sparrow, in fact their growth, with a consequent enlargement of the region in which they lie, causes the protruberance. According to Bullough (1942) these organs in the English starling consist of the much-coiled posterior ends of the vasa deferentia together with a number of blind branches of the vasa. He calls the organs seminal vesicles. He attributes their increase in size during the spring, not only to a lengthening and widening of the gonaducts, but also to an increase in the size and number of the blind branches. Witschi (1945) on the other hand, working with specimens of the same species introduced into America, finds no branches of the gonaducts and calls its coiled end a glomus. Webster's Dictionary defines a vesicle as 'a small bladder', and a glomus as 'a coil or small plexus'. In accordance with these definitions the findings of the present study agree with Witschi and are such as to cause the selection of the term 'seminal glomus' rather than 'seminal vesicle' for this organ in passerine birds.

In serial sections of the whole cloacal protruberance of the vesper sparrow and of the horned lark no branches of the gonaducts could be found. There are no junctions of tubules to be seen although such are often found in sections of the testes where the seminiferous tubules are commonly branched (Fig. 24). A technique was devised involving the projection of serial sections upon a screen so that the tubules could be followed by means of a continuous pencil line. This also showed a lack of branches in the ducts. Finally, parts of the coiled duct were teased apart and no branches could be found. Each organ then, appears as a much-coiled tube folded upon itself many times and forming a somewhat elliptical body which may best be termed a seminal glomus.

In the vesper sparrow the lumen of the glomeral duct varies from 150μ to 315μ in diameter when filled with spermatozoa, with an average diameter of about 250μ. There appears to be no regularity in the variation. Increase in diameter is probably caused by temporary congestion of semen in certain parts of the duct which becomes distended to accommodate it. There is evidence of stretching of the walls of the duct where the lumen is greatest.

The glomeral duct is lined with an epithelium averaging about 15 in thickness which consists mainly of a single layer of columnar cells whose vesicular nuclei are regularly arranged just below the middle of the cells (Fig. 19). Referring to the starling in a similar stage of development Bullough (1942) says that these cells 'were seen to be ciliated and actively secreting'. In the vesper sparrow the epithelium is not ciliated. However, numerous ragged strands extend a short distance into the lumen from many of the cells. This is taken as evidence of secretion and the epithelium is considered to be glandular. The presence of groups of cells pulled away from the tunica but still connected with it by short cytoplasmic threads indicates that desquamation takes place. It is possible that the secretion of the mucosa and the substance of the cast-off cells both serve a nutritive function in the semen.

Surrounding the columnar epithelium is a thin sheath of involuntary muscle about 7.5μ in thickness. Where the duct walls are very close together these muscle layers may lie in such close contact that they appear as a single layer but elsewhere they may be separated by a thin sheet of connective tissue. The interstices formed between the coils and folds of the glomeral duct are also filled with connective tissue, usually of the areolar type. In this run many capillaries.

The posterior end of each glomus is made up of several folds of the gonaduct whose lumen is regularly circular in cross-section and of an average diameter of about 225μ. Here the lining epithelium and its involuntary muscle tunica are of the same type and thickness as in the more anterior parts of the duct but connective tissue of the compact type forms a complete outer tunica at least 15μ in thickness. That part of this duct which lies at the extreme posterior tip of each of the glomera, commences a transition into the ejaculatory duct which folds back upon itself two or three times before appearing externally on the median surface of the glomus, as previously described. The first evidences of this change are a less regular structure of the mucosa and the appearance of a connective tissue tunica between it and the muscularis. Shortly after, the identity of the muscle tissue becomes obscured and no definite muscular tunic is evident. At first there is no increase in the diameter of the duct, in fact its lumen has been considerably reduced as the walls have thickened.

By the time the two ejaculatory ducts have reached the point at which they leave the glomera they have become well defined. Over a distance of 3 mm. each gradually swells to a diameter of about 750μ, becoming reduced again as it enters the urodaeum. Its lumen is small. Its mucosa consists of columnar cells but these are irregularly fitted together in more than one layer. The compactness of these cells and the fact that the mucosa is thrown into numerous longitudinal folds indicate that the duct is distensible. The connective tissue tunica becomes very thick and the muscle less evident. No definite muscular tunic can be seen.

The two ejaculatory ducts come close together on the dorsal surface of the urodaeum and open on two conical papillae

which project into its cavity (Fig. 11). These papillae are a short distance behind and above the openings of the ureters which are situated opposite each other on the sides of the urodaeum. The genital papillae project obliquely forward toward the copradaeum when the proctodaeum is in its normal position but when the proctodaeum is everted these papillae point backward toward the urodaeal outlet (Figs. 10 & 11). All three openings are thus brought into juxtaposition during coition.

Contents of the Duct

During the latter half of May and in June the whole gonaduct of the vesper sparrow is filled with semen. It contains spermatozoa so numerous that an estimate of their numbers would be subject to an error of millions. Stresemann (1934) states that one ejaculation in the pigeon contains two hundred millions. They are crowded together in haphazard fashion; only here and there are there groups showing orderliness by lying parallel with their tails sweeping in the same direction. Protoplasmic debris is found in varying amounts. Some of it probably descends from the testes, the bye products of spermiogenesis. Some of it, however, is the detritus resulting from the desquamation of the mucosa of the duct. Nuclear as well as cytoplasmic fragments may be found.

No attempt was made during this study to show any definite correlation between the nature of the semen and the breeding cycle. However, it was noted in the vesper sparrow that early in May the main constituent of the semen was debris with very few spermatozoa, typically occurring singly. At the height of the breeding season in June spermatozoa were most numerous and by the middle of July cellular debris again predominated. It was noted also that toward

the end of June spermatozoa with acrosomes missing appeared in increasing numbers, both in the duct and on sperm smears. The loss of the acrosome is the first step in the disintegration of spermatozoa.

Structure in Horned Lark

The cloacal protruberance of the horned lark differs from that of the vesper sparrow in size and shape. It is elongate, nearly cylindrical in form, but tapering slightly toward its distal end (Fig. 6). Its length when fully developed is about 9 mm. and its average diameter about 5mm. As in the vesper sparrow, the everted proctodaeum is very often exposed through the anal opening.

The bulbous type of cloacal protruberance was observed only on the vesper sparrow. The elongate type, or a slight modification of it, was found on the horned lark, Brewer's blackbird, redwing blackbird, and meadowlark. It has been observed by the writer previous to this study on a great variety of passerine birds including the spotted towhee, Pipilo maculatus, the robin, Turdus migratorius, McCown's longspur, Rhyncophanes mccowni, and many species of sparrows.

Differences in size and shape of the cloacal protruberance of different species are attributable to the size of the glomera and the amount of adjacent adipose and connective tissue. In the horned lark, a definite mass of fatty tissue is located between the glomera causing a much wider separation than was ever noted in the vesper sparrow. The glomera are much smaller in diameter than those of the vesper sparrow but about the same length, their shape being narrow ellipsoid (Fig. 8). They retain a more lateral position and were never noted to meet in a median line. This accounts for the cylindrical rather than spherical shape of the whole protruberance.

The glomeral duct folds and coils but does not branch. Its parts have an arrangement similar to those of the vesper sparrow. The lumen of the duct, however, is much smaller in diameter varying between 80μ and 210μ with an average of about 140μ. The mucosa averages a little thinner, about 13μ, and is more often cuboid than columnar. The involuntary muscle tunic is a little thicker, about 10μ or more in thickness.

No discernable difference was found in the ejaculatory ducts, nor in their openings into the urodaeum.

The contents of the ducts were similar to those of the vesper sparrow save only, of course, that the spermatozoa were quite different. These will be described below.

Discussion

The cloacal protruberance is formed in some passerine birds during periods of sexual activity. Witschi (Personal communication) assumed, without investigation, that it might be caused by a local endemous condition as well as by the growth and swelling of the seminal glomera. In the passerine birds examined during the course of this study such is not the case. The protruberance was found to be formed as a result of the enlargement of the glomera although sometimes there is also an accumulation of fatty tissue in their immediate vicinity. Naturally, there is some expansion of the muscular body wall to accommodate these enlarged organs, and there is some evidence to show that this muscular expansion occurs early, preceding rather than accompanying the enlargement of the glomera. However, the size of the protruberance is dependent mainly upon the size of the glomera. Witschi (1945) has shown that growth and regression of the gonaducts are controlled by gonadotropic hormones and that their cycle of development in the starling closely follows that of the testes. If we assume the same to be true in other

passerine birds then it follows that the development of the cloacal protruberance is linked with that of the testes.

So far, in experimental research on sex in birds, it has been found necessary to follow the sexual cycle by mutilating or sacrificing individuals from a flock of experimentals. This method of investigation has obvious disadvantages. Each specimen must be assumed to be a typical member of the group. It cannot be returned as a member of the experimental group since it is either harmed or dead. It is impossible to follow a series of internal changes in one individual. These disadvantages disappear when the necessity of mutilating or sacrificing the experimentals is removed. Witschi and Wood (1936) tacitly acknowledged this when they attempted to relate the color and size of the beak in the English sparrow with the development of the gonads. Any external feature whose measurable variations might serve as an indicator for the cyclical changes in the gonads warrants serious study.

Although the cyclical nature of the growth and regression of the cloacal protruberance was noted during the present investigation, the relation between this cycle and that of testicular growth was not studied. The migratory habits of the two major species present obvious difficulties to such a study by a resident worker. Certain impressions were gathered, however, with regard to this relationship. Foremost among these was the belief that the cycles of testicular and glomeral activity are not coincident, but that the former precedes the latter. Thus there is a lag of development of the cloacal protruberance behind that of the testes. An attempt was made during the early spring of 1948 to check this in the resident English sparrow, Passer domesticus. It was there found that the development of the seminal glomera very definitely lags behind that of the

testes. For example, on March 21 a male English sparrow showed nearly maximum growth of the testes (lengths, 9 mm and 10 mm.), yet the seminal glomera were very small (about 1.5 mm by 1 mm). It was interesting to note that the glomera contained completed spermatozoa in small numbers indicating maturity of the testes, yet it is doubtful that coition had occurred or that sufficient semen had been produced to accomplish successful fertilization. However, the cloacal protruberance had commenced development and was measurable although it was still small. If such a lag occurs in the horned lark indications were that it is not nearly as great as in the English sparrow.

Further work of a quantitative nature is anticipated which should show the feasibility of using the cloacal protruberance as an indicator of testicular activity.

SPERM SMEARS

Sperm smears were made from living or dead birds by placing a microscope slide against the anus in such a manner that the lips are opened against the glass, and then sliding it back toward the tail. Sometimes the faeces are extruded upon the first slide but they may be removed abd a second smear taken. Practically no pressure is required during this procedure. Very often a good smear is obtained immediately, especially at the height of the breeding season. At that time the proctodaeum is often exposed as a papilla at the anal opening and may be covered with semen. A mere touch of a slide against this surfacd is then sufficient to produce a good smear. If a positive smear was obtained easily in this manner the fact is indicated in Table II by the sign ✓✓✓.

If spermatozoa were not observed on smears made in the above manner, light pressure was applied on the cloacal protruberance with the thumb and fore-finger of the hand which held the bird and further smears were taken. A positive sperm smear obtained in this manner is indicated in Table II by ✓✓.

If neither of the above methods resulted ina positive sperm smear, greater pressure was applied, but only on dead birds, forceps sometimes being used to secure a sample of the semen. A positive sperm smear secured in this manner is indicated in Table II by ✓. Inability to secure spermatozoa in any of these manners is indicated in Table II by -.

Some sperm smears were dried and stored without further preparation; others were fixed in Bouin's solution and stained with either or both of Delafield's haematoxylin and aqueous eosin; others were stained without use of a fixative. The sperm smears were often greasy. Before staining, it was found best to remove fats by immersing the slide in xylol and then running it rapidly through alcohol

solutions from absolute through 95%, 85%, and 70%, before putting it in the aqueous stain solutions. Some were mounted in Canada balsam; others in glycerine. The most successful preparations were those which were put through the xylol and alcohol baths and dried immediately, unstained and unmounted; and those which were stained with Delafield's haematoxylin and eosin without the use of a fixative. Figure 20 shows an example of the first method of preparation. Figures 21 and 22 show examples of the second method.

The idea of taking sperm smears as evidence of full sexual maturity unfortunately did not occur to me until early in May, 1947. The first sperm smear from a horned lark was made on May 6th and thus the greater part of the first breeding period was missed. In the case of the vesper sparrow, however, a smear was taken from the first male collected during 1947 and from all succeeding males taken during that year. It is admitted that the number of specimens taken was too small, and the dates of collection too far apart, to draw very definite conclusions but Table II is offered as a possible indication of a trend. It lists the numbers, the dates of collection, and the ease with which sperm smears were obtained, of specimens of Otocoris alpestris and Pooecetes gramineus taken during 1947.

TABLE II

EASE OF OBTAINING SPERM SMEARS

* * *

Pooecetes gramineus

No.	Date	Method Obtained
112	May 7	-
113	May 9	-
121	May 14	/
123	May 17	/ /
126	May 21	/ /
129	June 1	/ / /
136	June 21	/ / /
139	June 28	/ / /
140	July 15	-

Otocoris alpestris

No.	Date	Method obtained
109	May 6	/ /
110	May 7	/ /
119	May 13	/
127	May 21	/
128	May 26	/ /
130	June 2	/
132	June 6	/ / /
133	June 15	/ / /
134	June 19	/
135	June 21	/
138	June 28	-

Table II shows that positive sperm smears were most readily obtained from vesper sparrows during the period from June 1 to June 28, a period which coincides well with the peak of testis development (Fig. 1). It also indicates that fertilization of eggs would be impossible before about May 14 and after July 15, and that the chances of fertilization before May 17 would be small.

In the horned lark positive sperm smears were obtained readily on May 6 and 7 toward the end of the first peak of testis-development (Fig. 1) but were difficult to secure a week later on May 13, and remained so, with one exception, until between June 6 and June 15 when positive smears were again easily obtained. This period coincides with the second peak of testis development. After June 19 sperms were obtained only with considerable difficulty. No sperms could be found on anal smears taken from a specimen collected on June 28, even after several attempts and the use of considerable pressure; yet the glomera were later found to contain a few spermatozoa. Successful fertilization is probably impossible after the end of June. No information was secured which would show the date at which fertilization could first be effected.

A very profitable and interesting problem arising from the above still remains to be carried out. It is quite probable that, in addition to devising a better method of measuring the ease of securing sperm smears, the vitality of the spermatozoa could also be measured. At very least, the presence of living or dead sperms could be observed. It is possible also that the nature of the semen as shown on the smear could be used as an indicator of the development of the testes. Thus experimental workers using passerine birds could with certainty tell the time at which sexual maturity is reached without sacrificing individuals, and could perhaps estimate the stage of development of the testes before or after

that time. Since the necessity of keeping large numbers of experimentals, which must be sacrificed from time to time and which may vary among themselves, has always been something of an obstacle to avian research on sex, this problem warrants consideration.

SPERMATOZOA

The descriptions of spermatozoa given below are of typical specimens observed on slides obtained by the first method described above under 'Sperm Smears'. Previous authors have described spermatozoa mainly from sections made of mature testis. Specimens obtained on sperm smears are ideal since they are known to be mature. In a well-made smaar the spermatozoa are distinct and straight. This makes them easy to observe and easy to measure. Facility in the technique of taking sperm smears is recommended for any student of avian spermatozoa.

Vesper Sparrow

The spermatozoon of the vesper sparrow has a long head and an exceptionally long tail. It is the longest spermatozoon encountered during the course of this study, sometimes reaching a total length of 245μ (Fig. 14 and Table III).

The head forms a spiral, the basal portion being thickened to contain the nucleus and then flattening rapidly to form a spiral membrane which is the acrosome. Apparently the nucleus is flattened somewhat to fit in the base of this spiral. When viewed from the side it is narrow and comma-shaped, curving with the trend of the acrosome (Fig. 12). In front view, however, it appears heart-shape

with an indentation anteriorly which gives a bifid appearance (Fig. 13). The nucleus is about 3μ in length and between 1μ and 1.5μ in greatest width.

The acrosome is a spiral membrane arising from the nuclear part of the head. Broad at its base, the membrane tapers off at the tip which is thus sharply pointed. It makes approximately two and one-half turns. The first turn is a fairly tight spiral throwing the membrane well beyond the width of its base; the next stretches out considerably; and the last part of the membrane travels far with very little turning. The average length of the acrosome is 13μ.

No well defined region which could be termed the neck was observed in any spermatozoa of the vesper sparrow, whether stained or unstained. However, the tail filament does not start abruptly from the posterior end of the nucleus. Instead there is a gradual tapering from one to the other. This conical structure probably represents the neck of this spermatozoon.

The tail is a very long protoplasmic thread arising from the posterior end of the neck. Starting here a narrow spiral membrane winds about this thread in drawn-out coils. The membrane disappears so gradually that its end and the beginning of the terminal filament were never definitely seen. The total length of the tail averages about 220μ.

Horned Lark

The spermatozoa of the horned lark are comparatively short, being only about one-third the length of those of the vesper sparrow (Fig. 14). The acrosome and tail are both shorter but the nucleus is about twice the length of that of the vesper sparrow (Fig. 16). Total length of the spermatozoon is about 86μ (Table III).

In stained specimens the head shows a long, stout nuclear region and a thinner, slightly shorter spiral acrosome. The acrosome is a spiral membrane not as wide at the nucleus as in the vesper sparrow, and gradually tapering to a point while making about two turns of a spiral which does not diverge very far laterally from a central axis. Its length is about 5μ.

The nucleus is a twisted, cylindrical mass whose diameter is about one-third of its length. It tends to follow the curvature of the spiral started by the acrosome. At its anterior end it is slightly concave where the acrosome joins it (Fig. 16). The length is about 6μ and the diameter about 2μ.

Attached posteriorly to the nuclear part of the head is the neck. It is quite short but correspondingly thin, its shape being long conical with the apex pointing posteriorly. The apical half stains darker than the basal portion in Delafield's haematoxylin. Its length is less than 1μ.

The tail of the spermatozoon is short. From the apex of the neck arises a filament around which turns a narrow membrane which gradually tapers off as it spirals. It makes approximately twenty turns over a distance of about 62μ before disappearing. The terminal filament continues on without this membrane to a length of about 14μ.

Discussion

During the breeding season of 1947 samples of spermatozoa were secured from four species of passerine birds. Among these spermatozoa those of the vesper sparrow were easily distinguishable, not only by the great length of the tail, but by the presence of the short stout nucleus which, under low magnification, appears

as a small dark sphere between the acrosome and the tail. The spermatozoa of Brewer's blackbird and of the cowbird are similar to each other and to those of the horned lark. In common they possess a fairly long twisted nucleus and a tail much shorter than that of the spermatozoon of the vesper sparrow. Despite their similarity, however, it was found possible to identify good specimens of any of them. Just how far the ability to identify a species from the size and shape of its spermatozoa could be developed was not further investigated.

After long examination of both stained and unstained sperm preparations the impression was gained that some step in the preparation of the smears brings about a change in the size and shape of the nucleus. The spermatozoon is one of the few places where such a change would be discernable. The change is believed to be similar to that which occurs in muscle upon application of extreme heat, or that which is found in a viscous liquid moving freely in space. Insufficient material was on hand during 1947 to permit experimentation which would prove or disprove this impression, but some measurements were made which strongly support it. The change is believed to occur in one or more of the following solutions: Bouin's fixative, acid alcohol, Delafield's haematoxylin. It is anticipated that further study of this problem during the avian breeding season of 1948 will bring about its solution.

TABLE III

LENGTHS OF SPERMATOZOA

		Vesper Sparrow	Horned lark
Head	Acrosome	13 ± .8 μ	5 ± .5 μ
	Nucleus	2.5 ± .5 μ	5.8 ± .2 μ
Tail	Spiral Membrane	220 ± 11 μ	62 ± 2 μ
	Terminal Filament		13.5 ± 1 μ
	Total Length	235 ± 12 μ	86 ± 4 μ

TESTIS

When this study was started during the spring of 1946 it was intended that a study of the testes of various passerine birds would form the major part of the work. It soon became apparent, however, that the testes of the species examined differed in no important respects from those of several species of birds already reported upon by workers better qualified than the writer. An examination of other parts of the reproductive tract suggested more interesting and profitable problems and a search of the pertinent literature showed that much less work had been done upon the accessory sexual organs. Consequently it was decided to relegate a study of the testes to a position of minor importance.

The testes of all birds collectdd were preserved, sectioned, and examined. The following observations were made during the course of these examinations. They do not pretend to be a comprehensive study of the testes of passerine birds but are considered to be of sufficient importance to record.

Testis Size of Horned Lark

The horned lark was chosen as one species for study partly because in southern Alberta it produces two broods per year, one hatched early in May, the other late in June. Measurements were taken of testis sizes of specimens collected during the entire breeding season. It was noted that the shapes of the testes were particularly uniform within the series, the right always being practically spherical, the left ovoid, with the ratio of major to minor axis approximately 3:2. Since the volume of a regular body is proportional to any of its linear measurements the length of such a body is as good an indicator of variation as the volume. Therefore,

lengths of the testes were chosen as the standard of measurement. The accompanying graph (Fig. 1) uses average lengths of the two testes as a basis of comparison of specimens. It shows a composite picture of all specimens taken during 1946 and 1947. At that it is recognized that the data are too meagre. However, since there was no purposive selection of material it might be assumed that the specimens collected were representative ones and that the graph is significant. A graph of testis sizes of the vesper sparrow which breeds but once per year in southern Alberta is included for comparative purposes.

The most striking feature of this graph is the double crest. Testes of the horned lark increase in size during March and April, reaching a peak about the time that the first set of eggs is being incubated. Regression then occurs for about two weeks after which further increase in size for about twenty-five days brings them back to a second peak at a time when the second set of eggs is in the nest.

Keck (1934) gives a graph of testis size of the English sparrow, Passer domesticus, which shows a concavity at the top suggestive of a double peak. Bullough (1942) by-passes this subject in the English starling, Sturnus vulgaris, by giving only monthly averages of testis volumes. Another worker with the starling, Witschi (Personal communication), has no records from which deductions might be drawn. These are the only three passerine species, so far studied, which regularly breed more than once per year. It is noteworthy that two of the three species show a period of testis regression between periods of sexual activity.

It is not intended to enter into the controversial discussion of the various external stimuli which bring about recrudescence and regression of the avian testis, but merely to point out the signif-

icance of the graph when considered in relation to the more commonly proposed stimuli; viz., variation in day-length, in temperature, and in activity.

It is noted that both regression and recrudescence, involving a variation of about 2 mm. in testis length of horned larks, occur during a period when day-length and average temperature are increasing. It is also noted that regression of the testes follows a period of relative activity of the male while the female is incubating, and that recrudescence follows a period of great activity during which he assists in ministering to the needs of a rapidly growing family. The 'lag' in each case is approximately ten days. Apparently such a lag of response behind stimulus is normal, for Bissonnette (1931), using experimental increases of day-length on starlings, found that after five days the testes were not affected but after seven days they were 'little enlarged'. Assuming a similar lag for horned larks in this instance, it would appear that the facts fit the activity theory very closely.

This same topic, examined in some species which breeds twice annually and in which the male assists in the duty of incubation thus becoming relatively sedentary between breeding periods, would make an interesting subject for further research.

An Example of Physiological Separation of Subspecies

Since the writer personally collected and prepared all materials for study in this thesis there was ample opportunity for recognition and study of problems other than those directly connected with anatomy and histology. The following note is considered of sufficient importance to warrant its inclusion in this paper. It is andefinite illustration, of which few have been recorded, of what has been called 'the physiological differentiation of subspecies'.

On April 18, 1947, a male desert horned lark, Otocoris alpestris leucolaema, was collected from a field of alfalfa in which a few pairs of birds of this subspecies had obviously chosen territories and nesting sites. The testes of this bird measured 7 mm. and 9 mm. in length. Later examination showed mature spermatozoa in the tubules of the testes (Fig. 24) and the seminal glomera were filled with spermatozoa. In other words there seems to be no doubt that it was a breeding bird.

A few moments after this bird was collected attention was attracted to a flock of horned larks feeding in a fallowed field across the road and about one hundred and fifty yards distant. Their presence as a flock, when horned larks should be building nests, was unusual and a specimen was collected. This flock, or others with numbers varying from twenty-five to seventy-five, was present on the following two days, and further specimens were secured, a total of seven, of which six were males. All were Hoyt's horned lark, Otocoris alpestris hoyti, a darker subspecies than the resident desert horned lark. On April 21 they were gone and it was assumed that the birds had continued their migration northward.

TABLE IV

TESTIS LENGTHS OF OTOCORIS ALPESTRIS HOYTI IN MIGRATION

No.	Date	Testis Length		
		Left	Right	Average
98	April 18	2.75 mm.	2 mm.	2.38 mm.
99	April 19	2.5	2	2.25
100	April 19	2.5	2	2.25
101	April 19	2	1.5	1.75
102	April 19	3	2	2.5
105	April 20	2.5	5	3.75
				2.48

Table IV shows the lengths of the testes of the six males collected. The average length is about 2.5 mm. at a time when the testis-length of the locally bredding desert horned larks is 8 mm. or more (Fig. 1). Sections of the testes show development of the germ cells in each case. Spermatogonia are present in all testes and perhaps also primary spermatocytes although these latter in the resting stage are sometimes difficult to distinguish from spermatogonia. Primary spermatocytes in the synizesis phase were numerous in the testes of Nos. 98 and 105 (Fig. 25). No stages of spermatogenesis beyond this were noted in any of these testes. None of the six males was in breeding condition. The female was in an equivalent stage of development.

Here then are two subspecies of horned lark present in the same region during the spring of the year. Despite their proximity and their close relationship they do not intermingle. Their behaviorisms are markedly distinct. In one, Otocoris alpestris leucolaema, the gonads are fully developed; in the other, Otocoris alpestris hoyti development is slight. Due to this disparity in sexual development the former has completed its migration, chosen a territory, and is breeding: while the latter is still gregarious and migratory. They are physiologically incapable of inter-breeding. A physiological condition separates them while contiguous, as effectively as any geographic barrier could do, and by the time this difference in sexual development has become equalized the original disparity has caused a geographic barrier to be placed between them. These appear to be as good grounds as any on which to base subspecific distinction.

It is my opinion that many of the avian subspecies with north-south distribution evolved as a result of original minor

variations in rate of sexual development with consequent variation in migration distance. The effects were cumulative within the environmetal limitations of the species. Superficial distinctions, not necessarily adaptive, in size, color, etc., followed in response to different environmental factors, and subspecific identity was established.

It is believed that, within many species with wide north-south distribution, the whole population does not migrate as a phalanx with fixed relative positions, but that the members at the extremeties hop over the medial ones which are inclined to remain sedentary. This has been demonstrated in certain hawks (Salt, 1939) and is at present under study by the writer in waterfowl. Here it is demonstrated in the horned lark. Such habits tend to produce and fix geographically a variety of subspecies. Where the front of the migration is wide the number of subspecies may be greatly increased. Such appears to be the case in the horned lark.

Sertoli Cells

A close inspection was made of testis sections of all species collected with the express purpose of discovering the presence of Sertoli cells. None were observed. Mature spermatozoa are present in the seminiferous tubules in groups, very regularly arranged in passerine species (Fig. 24), less so in non-passerine species. No positive evidence was discovered, however, which would point to the presence of any type of 'mother cell' in connection with these groups.

SUMMARY

1. An external feature, the cloacal protruberance, is discovered on some species of passerine birds.
2. A description is given of this protruberance as observed on the vesper sparrow, Pooecetes gramineus, and on the horned lark, Otocoris alpestris.
3. The histology of the accessory sexual organs found in this protruberance is described.
4. Methods of obtaining sperm smears from some passerine birds are given.
5. The spermatozoa of the vesper sparrow and the horned lark are described.
6. It is suggested that the size of the cloacal protruberance and the nature of the semen obtained on sperm smears might be used as indicators of testicular activity.
7. A double peak is found in the spring cycle of testis development of the horned lark, and an explanation of this in terms of the activity theory is attempted.
8. An example of the physiological separation of subspecies is recorded from disparities of gonad development found in the desert horned lark and Hoyt's horned lark.
9. An apparent absence of Sertoli cells is noted in the mature testes of certain passerine birds.

BIBLIOGRAPHY

Benoit, Jacques (1936) - Stimulation par la Lumiere de l'Activite sexuelle chez la Canard et la Cane domestiques:
Bull. Biol. Tome LXY Fasc. 4.

Bent, A.C. (1942) - Life Histories of North American Flycatchers, Larks, Swallows, and their Allies:
U.S. Nat. Mus. Bull. 179.

Bissonnette, T.H. (1930) - Studies in the Sexual Cycles of Birds, Parts I and II:
Am. Jour. Anat. Vol. 45, No.2.

(1931) - Studies in the Sexual Cycles of Birds, Part III: Am. Jout. Anat. Vol. 46, No.3.
Part IV: Jour. Expr. Zool. Vol. 58, Jan.

(1936) - Sexual Photoperiodicity:
Jour. Her. Vol. 27, No. 5.

(1936) - Fertile Eggs from Pheasants in January by Night-lighting:
Bird-Banding, Vol. VII, No. 3.

(1936) - Sexual Photoperiodicity:
Quart. Rev. Biol. Vol. II, No. 4.

Bissonnette, T.H. & Wadlund, A.P.R. (1931) - Spermatogenesis in Sturnus vulgaris.
Jour. Morph. & Phys. Vol. 52, No. 2.

Bissonnette, T.H. & Zuyko, A.J. (1936) - Normal Progressive Changes in the Ovary of the Starling (S. vulgaris) from Dec. to April:
Auk, Vol. LIII, Jan.

Blanchard, B.D. (1941) - White Crown Sparrows (Z. leucophrys) of the Pacific Seaboard: Environment and Annual Cycle:
U. of Cal. Pub. in Zool. Vol. 46, No. 1.

Bullough, W.S. (1942) - Reproductive Cycles of the British and Continental Races of the Starling:
Phil. Trans. Roy. Soc. of Lon., Ser. B, Biol. Sc. Vol. 46, No. 1.

Crew, F.A.E. (1926) - On Fertility in Domestic Fowl:
Proc. Roy. Soc. Edin. Vol. XLVI, Part II, No 20.

Gadow & Selenka (1891) - Anatomie der Vogel: Leipzig.

Gibbs, C.S. (1935) - A Guide to Sexing Chicks:
Orange Judd Pub. Co.

Hohn, E.O. (1947) - Sexual Behavior and Seasonal Changes in the Gonads and Adrenals of the Mallard:
Proc. Zool. Soc. Lon. Vol. 117, Part II.

Keck, W.N. (1934) - The Control of the Secondary Sex Characters in the English Sparrow, Passer domesticus (Linn):
Jour. Exp. Zool. Vol. 67.

Miller, R.A. (1938) - Spermatogenesis in a Sex-reversed Female and in a Normal Male of the Domestic Fowl, Gallus domesticus:
Anat. Rec. Vol. 70, No. 2, Supp. 2.

Moreau, R.E., Wilk, A.L. & Rowan, W. (1947) - The Moult and Gonad Cycles of Three Species of Birds at Five Degrees South of the Equator:
Proc. Zool. Soc. Lon. Vol. 117, Pt. II.

Retzius, Gustav - Die Spermien der Vogel:
Biol. Unt. N.F.B. XIV, 10, Plates.

Riddle, O. (1927) - Cyclical Growth of Vesicula Seiminalis in Birds is Hormone Controlled:
Anat. Rec. Vol. 1, No 2.

Riley, G.M. (1936) - Light Regulation of Sexual Activity in the Male Sparrow (Passer domesticus):
Proc. Soc. Exp. Biol. & Med. No. 34.

(1937) - Experimental Studies on Spermatogenesis in the House Sparrow, P. domesticus:
Anat. Rec. Vol. 67. No. 3.

(1938) - Cytological Studies on Spermatogenesis in the House Sparrow, P. domesticus:
Cytologia, Vol. 9 (2/3).

(1940) - Light vs Activity as a regulator of the Sexual Cycle in the House Sparrow:
Wilson Bull. Vol. 52, No. 2.

Rowan, W. (1926) - Notes on Alberta Waders included on the British List: Pt. IV
British Birds, Vol. XX.

(1929) - Manipulation of the Reproductive Cycle: Seasonal Histological Changes in the Gonads:
Pro. Bos. Soc. Nat. Hist. Vol. 39, No. 5.

(1938) - Light and Seasonal Reproduction in Animals:
Biol. Rev. Vol. 13.

Rowan, W. & Batrawi, A.M. (1939) - Comments on the Gonads of Some European Migrants collected in East Africa Immediately Before their Spring Departure:
Ibis. Jan. 1939.

Salt, W.R. (1939) - Notes on Recoveries of Banded Ferruginous Roughleg Hawks (Buteo regalis): Bird Banding, Vol. X, No. 2.

Stresemann, E. (1934) - Handbuch der Zoologie: Vol. VII. Pt. 2, Sauropsida: Aves.

Witschi, E. (1935) - Origin of Asymmetry in the Reproductive System of Birds: Am. Jour. Anat. Vol. 56, No.1.

(1935) - Seasonal Sex Characters in Birds and their Hormonal Control: Wil. Bull. Vol. XLVII.

(1945) - Quantitative Studies on the Seasonal Development of the Deferent Ducts in Passerine Birds: Jour. Exp. Zool. Vol. 100, No. 3.

Witschi, E. & Woods, R.P. (1936) - The Bill of the Sparrow as an Indicator for the Male Sex Hormone Jour. Exp. Zool. Vol. 73.

FIGURE I

Spring Testis Size of *Otocoris alpestris leucolaema* and *Pooecetes gramineus*.

Empirical graph showing, in black, testis lengths of specimens of *Otocoris a. leucolaema* collected during period April to June of 1946 and 1947.

And. in red, testis lengths of specimens of *Pooecetes grammineus* collected during period May to June of 1946 and 1947.

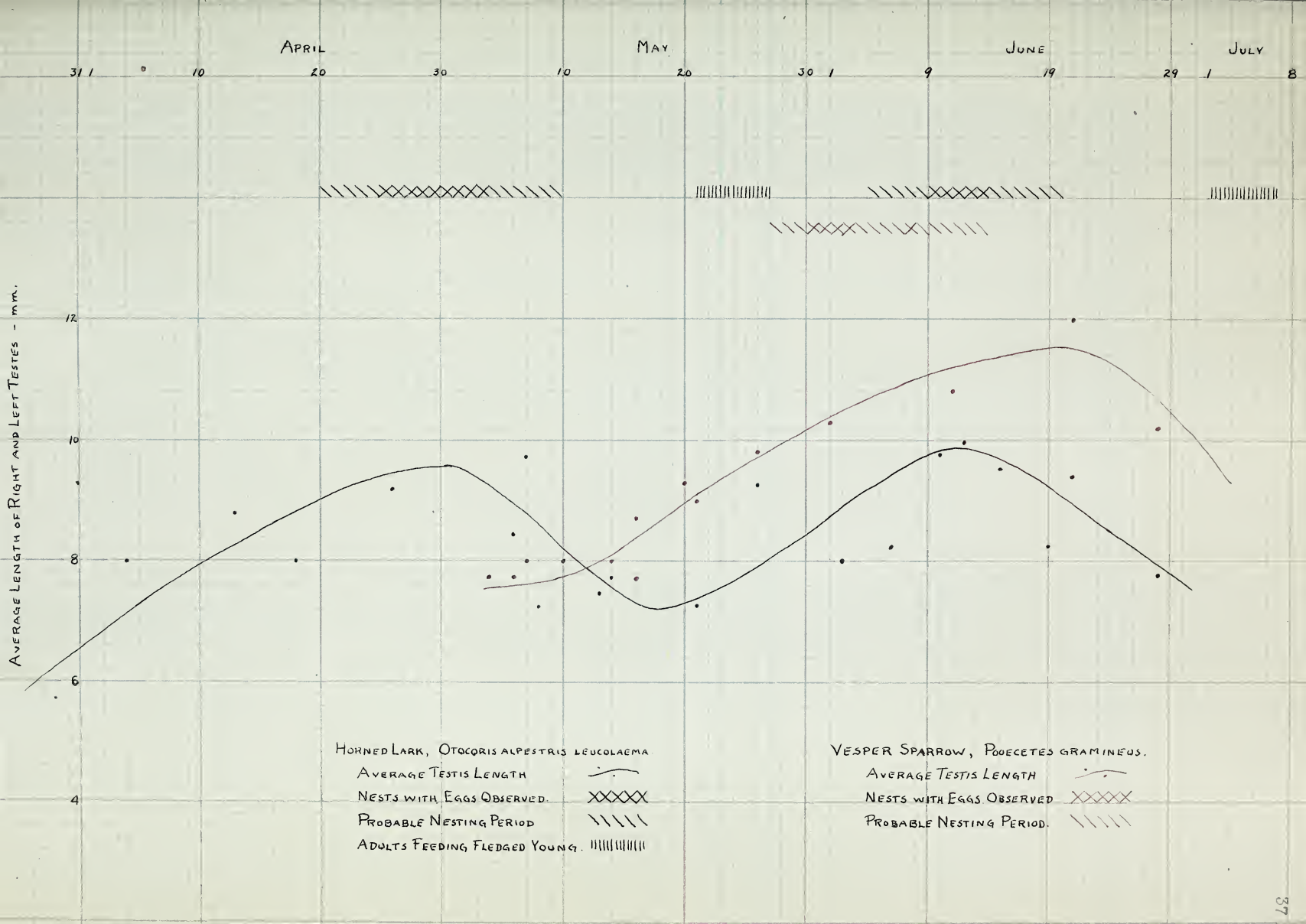
April
May
June
July
31
1
10
20
30
10
20
30
1
9
19
29
1
8
Average Length of Right and Left Testes - mm.
12
10
8
6
4
Horned Lark, Otocoris alpestris leucolaema
Average Testis Length
Nests with Eggs Observed.
Probable Nesting Period
Adults Feeding Fledged Young.
Vesper Sparrow, Pooecetes gramineus.
Average Testis Length
Nests with Eggs Observed
Probable Nesting Period.

PLATE I

CLOACAL PROTRUBERANCES

Figure 2 - Cloacal Protruberance of male *Pooecetes gramineus*, No. 121, May 14, 1947. X2.5

(No. 140, July 15, 1947, similar)

Figure 3 - Cloacal protruberance of male *Pooecetes gramineus*,

No. 129, June 1, 1947. X2.5

Figure 4 - Anal region of male *Pisobia melanotus*,

No. 124, May 17, 1947. X2.5

Figure 2

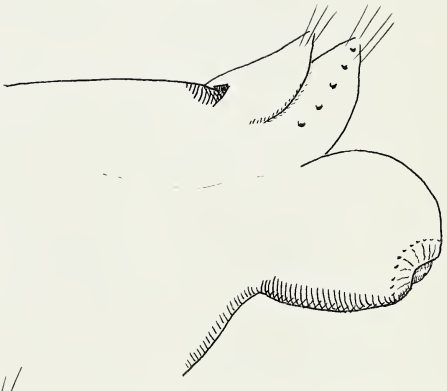

Figure 3

Figure 4

PLATE II

CLOACAL PROTRUBERANCES

Figure 5 - Cloacal Protruberance of male *Otocoris alpestris*,

No. 141, March 20, 1948. X4

Figure 6 - Cloacal Protruberance of male *Otocoris alpestris*,

No. 135, June 21, 1947. X4

Figure 7 - Anal Region of female *Otocoris alpestris*,

No. 144, March 20, 1948. X4

Figure 5

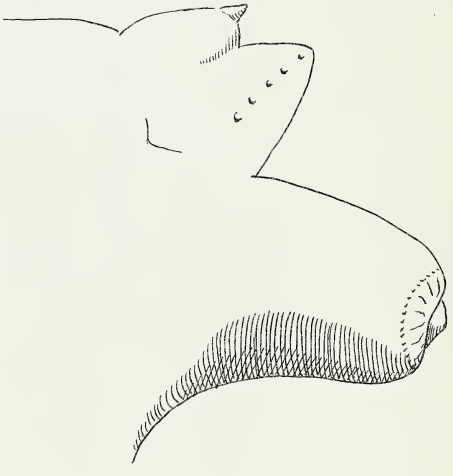

Figure 6

Figure 7

PLATE III

REPRODUCTIVE SYSTEM

Figure 8 - Gonads and Gonaducts of male Otocoris alpestris, No. 127, May 21, 1947. (Rectum displaced ventrally and to left; left testis slightly raised.)

T	- Testis	M	- Metanephros
E	- Epididymis	U	- Ureter
V	- Vas deferens	R	- Rectum
G	- Seminal glomus		

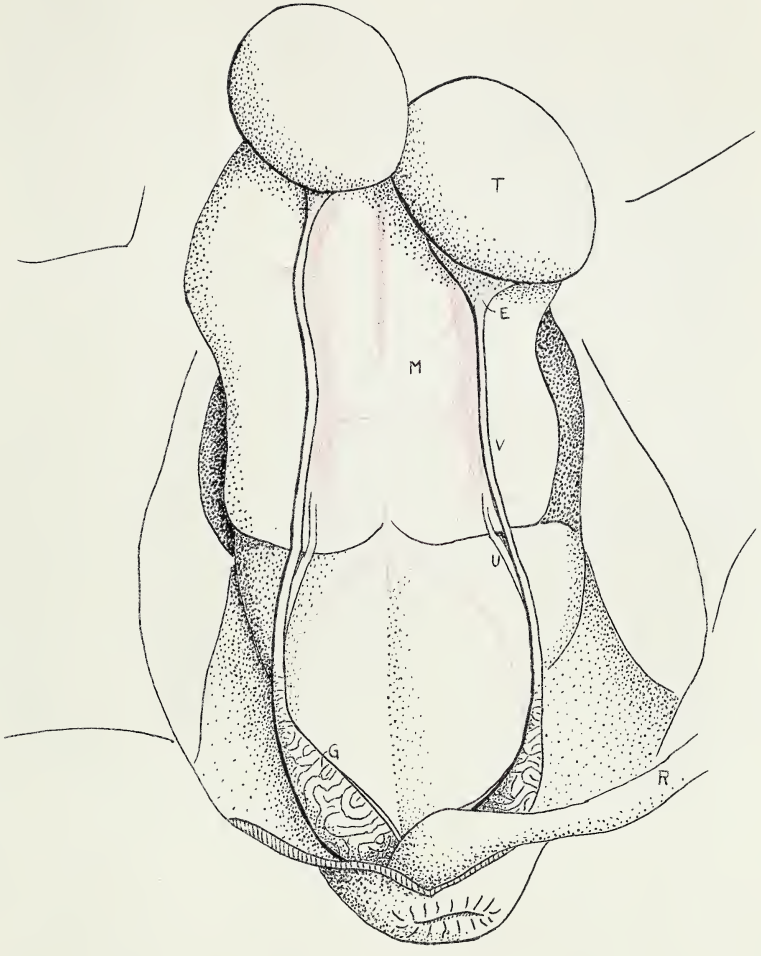

Figure 8

PLATE IV

CLOACAL PROTRUBERANCE

Figure 9 - Dorsal Dissection of Cloacal Protruberance of male *Pooecetes gramineus*, No. 129, June 1, 1947. Semi-diagramatic. (Rectum displaced to right; some fatty tissue removed from left side.)

V - Vas deferens
G - Seminal glomus
E - Ejaculatory duct
P - Proctodaeum
R - Rectum
C - Cloaca
U - Ureter

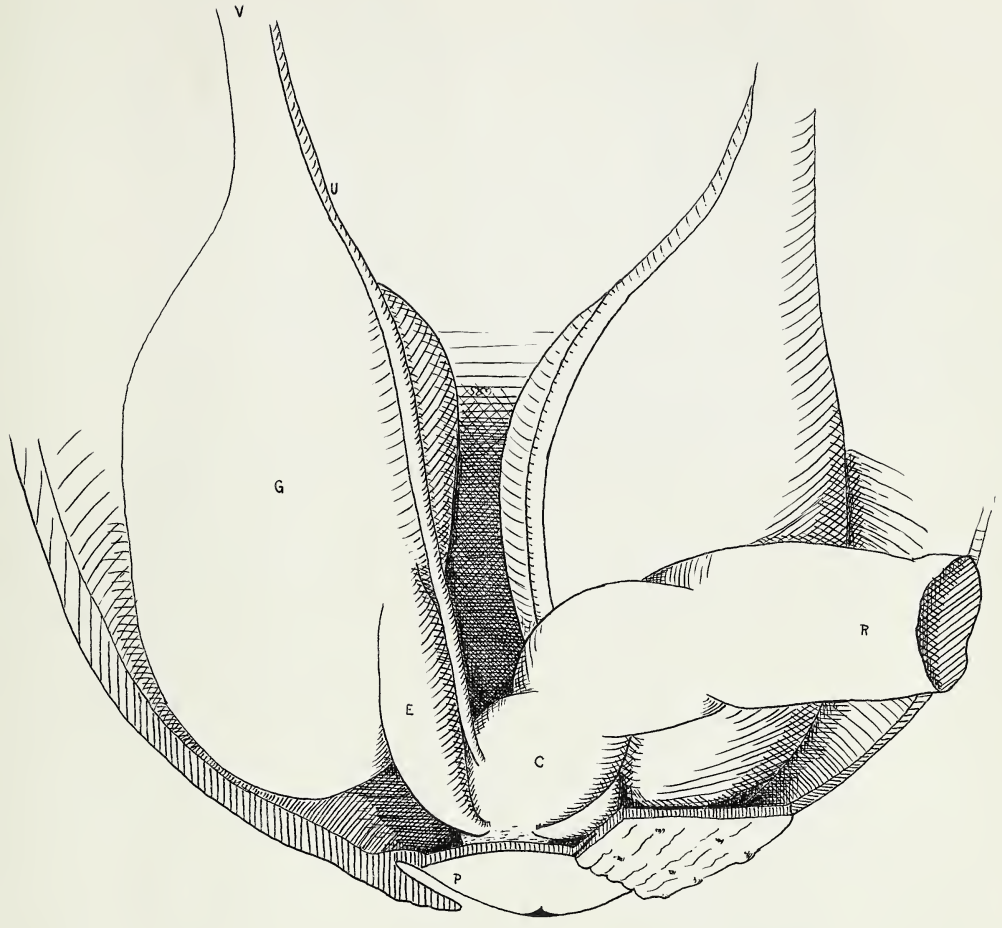

Figure 9

PLATE V

CLOACAL PROTRUBERANCE

Figure 10 - Right Half of Cloacal Protruberance of male Otocoris alpestris, cut slightly to left of mid-line. Semi-diagramatic. Ureter omitted, Proctodaeum inverted.

C - Copradaeum
Ur - Urodaeum
Pd - Proctodaeum
E - Ejaculatory duct
P - Genital papilla
U - Urinary papilla
G - Seminal glomus

Figure 11 - Right Half of Cloacal Protruberance of male Pooecetes gramineus, cut on mid-line. Semi-diagramatic. Ureter omitted, Proctodaeum everted.

Lettering as in Figure 10.

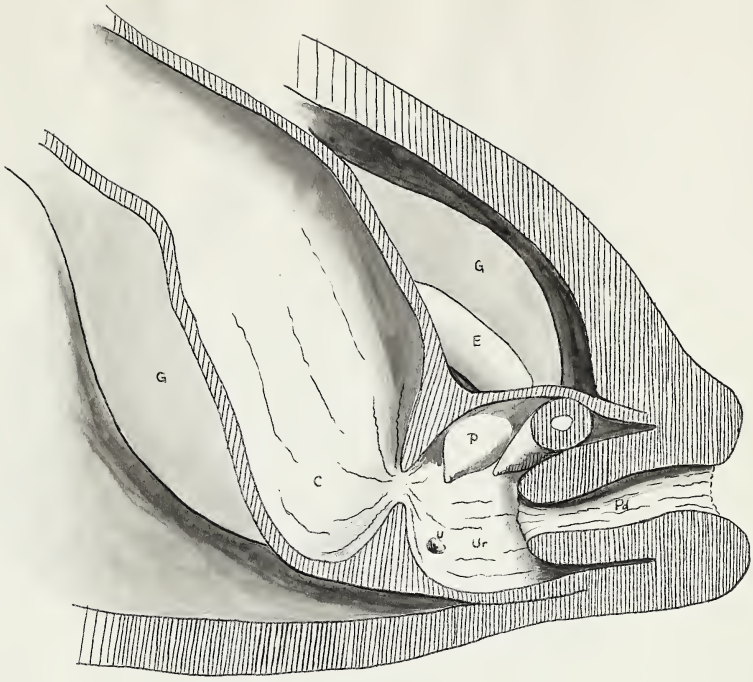

Figure 10

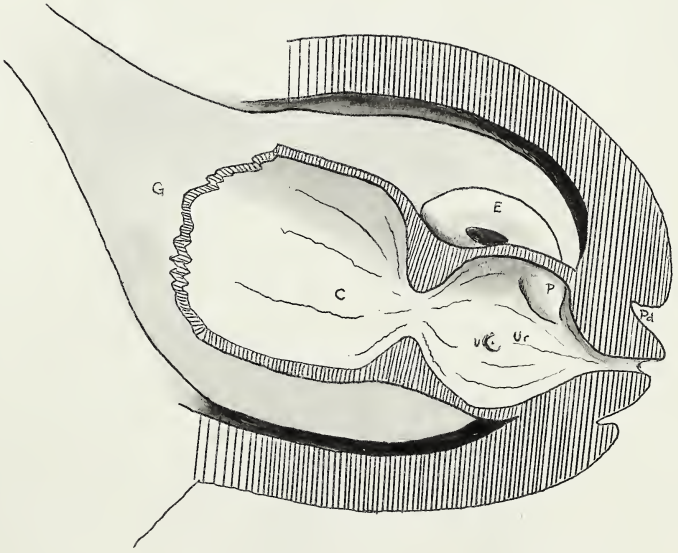

Figure 11

PLATE VI

SPERMATOZOA

Figure 12 - Head of Spermatozoon of *Pooecetes gramineus*, side view.

Figure 13 - Head of Spermatozoon of *Pooecetes gramineus*, face view.

Figure 14 - Spermatozoon of *Pooecetes gramineus*.

Figure 15 - Spermatozoon of *Otocoris alpestris*.
(Same scale as Fig. 14.)

Figure 16 - Head of Spermatozoon of *Otocoris alpestris*.
(Same scale as Fig. 12 & Fig. 13.)

Fig. 12

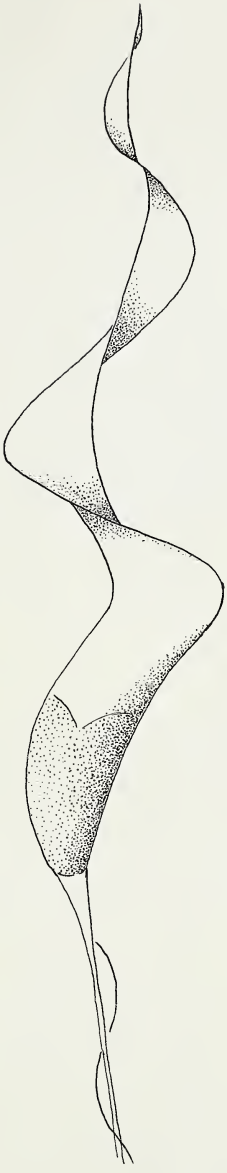

Fig. 13

Fig. 14.

Fig. 15

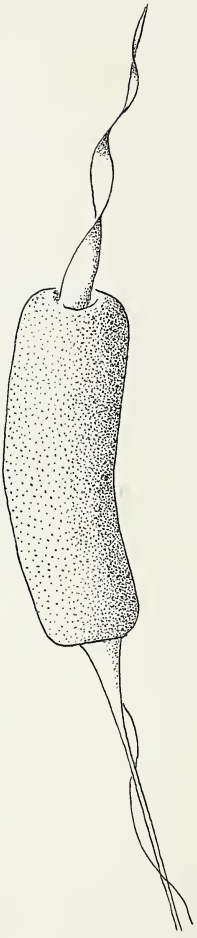

Fig. 16

PLATE VII

CLOACAL PROTRUBERANCE

Figure 17 - Cloacal Protruberance of male *Otocoris alpestris*, March 29, 1947. Photomicrograph of Oblique Section toward Posterior End of Glomera. Ejaculatory Ducts Visible.

Figure 18 - Cloacal Protruberance of male *Poecetes gramineus*, June 21, 1947. Photomicrograph of Oblique Section to show Openings of Ejaculatory Ducts on Papillae in Urodaeum.

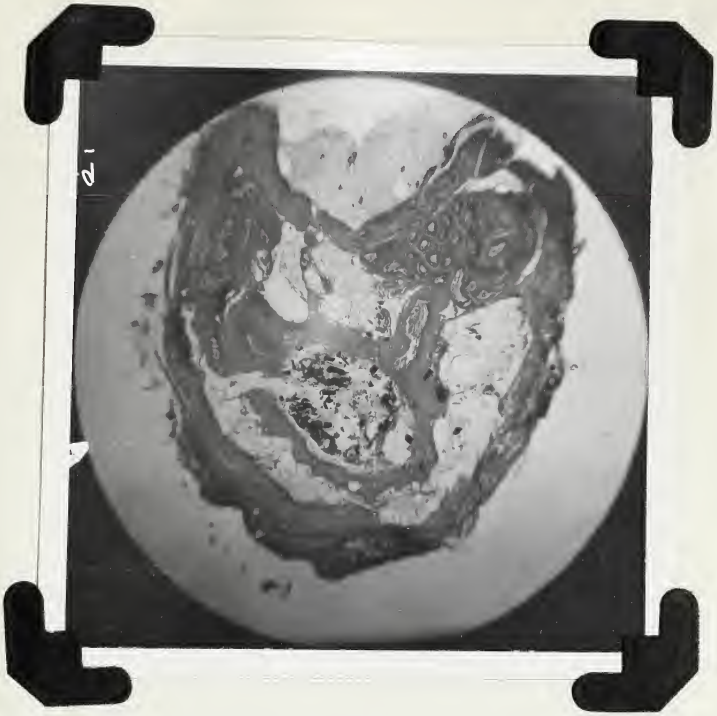

Figure 17

Figure 18

PLATE VIII

CLOACAL PROTRUBERANCE

Figure 19 - Ducts in Glomus of male Pooecetes gramineus, June 21, 1947. Photomicrograph to show Duct Walls and Contents of Ducts.

X440

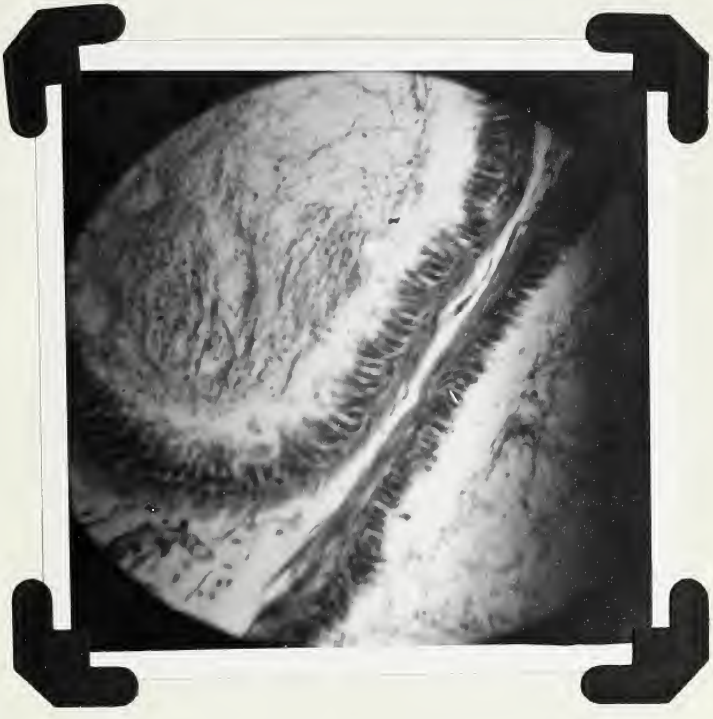

Figure 19

PLATE IX

SPERMATOZOA

Figure 20 - Spermatozoa of Pooecetes gramineus. Photomicrograph of dry sperm smear, unfixed and unstained. X900

Figure 21 - Spermatozoa of Pooecetes gramineus. Photomicrograph of Sperm Smear, Stained with Delafield's Haematoxylin and Eosin. X900

Figure 20

Figure 21

PLATE X

SPERMATOZOA

Figure 22 - Spermatozoa of Otocoris alpestris.
Photomicrograph of Sperm Smear
Stained with Delafield's Haematoxylin
and Eosin. X440

Figure 23 - Spermatozoa of Otocoris alpestris.
Photomicrograph of Sperm Smear
Stained with Delafield's Haematoxylin
and Eosin. X900

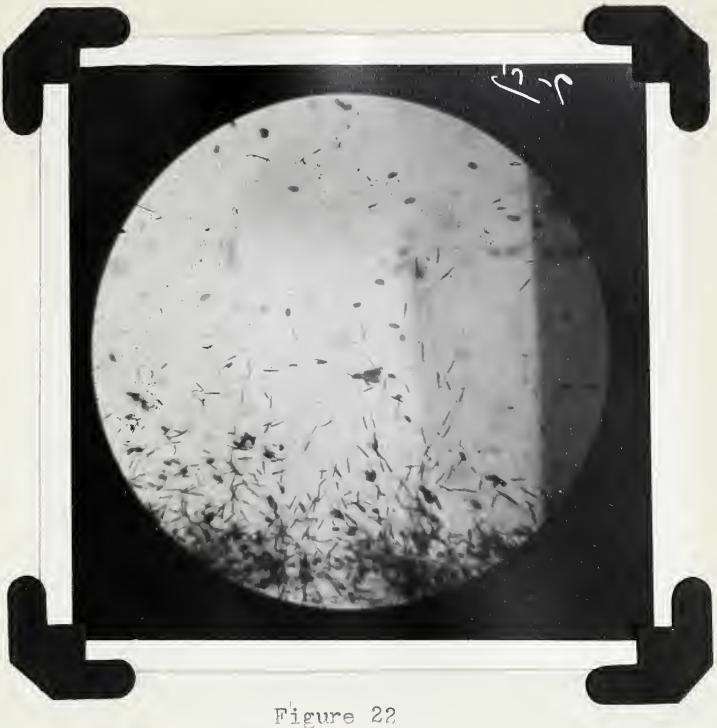

Figure 22

Figure 23

PLATE XI

TESTIS

Figure 24 - Section of Testis of *Otocoris alpestris leucolaema*, No. 97, April 18, 1947. Photomicrograph of section of Testis to show Mature Spermatozoa and Branching of Tubules. X100

Figure 25 - Section of Testis of *Otocoris alpestris hoyti*, No. 98, April 18, 1947. Photomicrograph of section of Testis to show Primary Spermatocytes in Synizesis Phase. X440

Figure 24

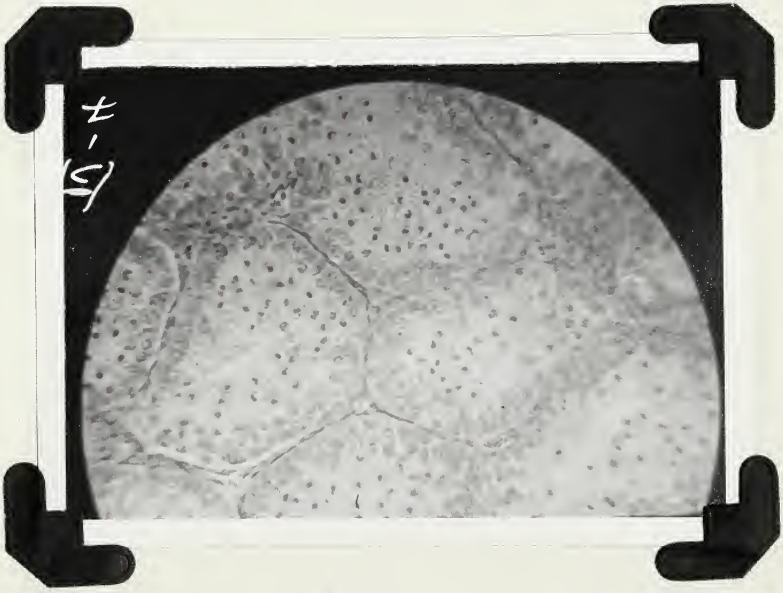

Figure 25

B29755